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Chapter 19
Role of entomopathogenic fungi in biocontrol of insect pests

Abstract: Chemical pesticides have an adverse impact on non-target organisms, and it leads to biodiversity loss, loss of food safety, development of insect resistance and resurgence in newer areas. All these have led scientists to create more ecofriendly alternatives, such as the use of entomopathogenic fungi against insect pests. Entomopathogenic fungus is a promising alternative to chemical insecticides that provides biological plant protection against insect pests in a sustainable pest control approach. Insect-infecting fungi are now classified into 90 genera and roughly 800 entomopathogenic fungal species have been documented. However, most commercial mycoinsecticides target just three genera: *Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosoroseus*. They cause about 60 percent of insect diseases. These fungi are key contributors to soil insect population dynamics. Hence, entomopathogenic fungi are important biocontrol agents against insect populations. Insect-infecting fungi are found in several distinct groupings. Insect fungal pathogens include those from the phyla Chytridiomycota, Zygomycota, Oomycota, Ascomycota, and Deuteromycota, which are known to be the best entomopathogens against various insect pests. Entomopathogenic fungi kill or inactivate insects by attacking and infecting their insect hosts. Entomopathogenic fungi are soil-dwelling fungi that infect and kill insects by breaching their cuticle. Most insect-infecting fungi work through penetration. Entomopathogens produce these extracellular enzymes (protease and lipase) and toxins in their adaptive response. Together with a mechanical process via appressoria growth, these enzymes break the insect cuticle and enter the body of the insect to infect and kill it by getting their nourishment from the insect tissues. On the other hand, insects have developed many defense against these fungal pathogens. Insect pests are effectively killed by the soil fungus, *Beauveria bassiana*, and are easy to use in the field. Now mass manufacturing of new fungal formulations are possible. Further, modern genetic engineering and biotechnology approaches may assist in increasing the bioactivity of entomopathogenic fungi. This chapter discusses entomopathogenic fungi and their detailed usage description in the current scenario. It also explains the mode of infection, approaches, plans, and policies for entomopathogenic fungi.

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19.1 Introduction

Insects are omnipresent, vast in their diversity, and make up almost three-quarters of all living species. Insects make for 4–8 million of the estimated 5.57–9.8 million animal species worldwide. They are exploited in the manufacture of silk, honey, lac, medicinal entomological medications, forensics, and biocontrol agents [1]. At the same time, they are potentially responsible for damaging crops, humans, and livestock, mostly as a vector for pathogens. Pest control is advancing with modern technologies while remaining eco-friendly. This led to the employment of biological species, particularly microorganisms such as bacteria, fungi, protozoa, and viruses, generally referred to as entomopathogens, to manage such insects. Entomopathogens, rather than broad-spectrum insecticides, can be employed to control insects. They contribute to the natural control of arthropod population and are preferable to pesticides in many instances. In addition to their efficiency, using entomopathogens has several advantages. These include preservation of natural enemies, reduced pesticide residues in food, and greater biodiversity in a controlled environment [2], and can be used in various ways, including as beneficial PGPRs and biofertilizers, in addition to their traditional role as bio-insecticide [3]. Fungi, as entomopathogenic, have a wider range of hosts and can infect both above-ground and underground pests, including soil-dwelling nematodes, and are most advisable for managing the pests of the soil, whereas the bacteria and viruses are more specific to their host [4]. This is one of the reasons why entomopathogenic fungi are preferred. Various fungi are entomopathogens. They range from severe specialists with restricted host ranges to generalists with relatively broad host ranges [5]. As both living and resting spores, fungi survive in the environment and infect insects when they contact them. Entomopathogenic fungi do not kill all insects but rather maintains their population below the economic threshold level. Entomopathogenic fungi are commercially cultured on a large scale as interest in these fungi is gradually increasing among the farmers. Over 170 different strains of mycopesticides are now used in commercial applications [3]. Several research studies are also being carried out everyday in this field to ensure its efficiency and for more beneficial exploitation. This chapter will give a brief summary of entomopathogenic fungi, their classification, mode of action, formulations, and finally, the new policies and plans for importing and exporting them.

19.2 Classification of entomopathogenic fungi

Among the living organisms, fungi range between 1.5–5.1 million globally. Fungi invading the dead and living insects are classified as saprophagous and entomophagous, respectively [6]. Entomophagous fungi consist of 100 genera. Out of the 100 genera, 750–1000 species are entomopathogens [6], [7]. Recently, insect-infecting

fungi have been discovered in more than 700 species and 90 genera [8–10]. Entomopathogenic are categorized into 12 groups and six phyla within the kingdom fungi [11]. In these six phyla, true fungi are categorized into four phyla, namely, Basidiomycota, Ascomycota, Zygomycota (subphylum: Entomophthoromycotina), and Chytridiomycota. Artificial phylum Deuteromycota, which includes a filamentous fungus that exists in asexual forms (anamorph) [12] and the newly discovered Glomeromycota are the only phylum in which entomopathogens are not present [13]. Most entomopathogenic fungi that are discovered belong to the Zygomycota (class Entomophtohorales) and Deuteromycota (class Hyphomycetes). Entomopathogenic fungi never form one monophyletic group. Thus, 12 Oomycetes species, 65 Chytridiomycota species, 339 Microsporidia species, 474 Entomophthoramycota species, 238 Basidiomycota species, and 476 Ascomycota species have been reported [14]. Alternaria, Cladosporium, Aspergillus, and Penicillium are the common fungi found in insect cadavers. Insect orders affected by entomopathogenic fungi include Diptera, Lepidoptera, Orthoptera, Hymenoptera, Coleoptera, and Hemiptera [15]. The classification of phylum is shown in Figure 19.1. Classification of various entomopathogenic fungi and their family are listed in Table 19.1.

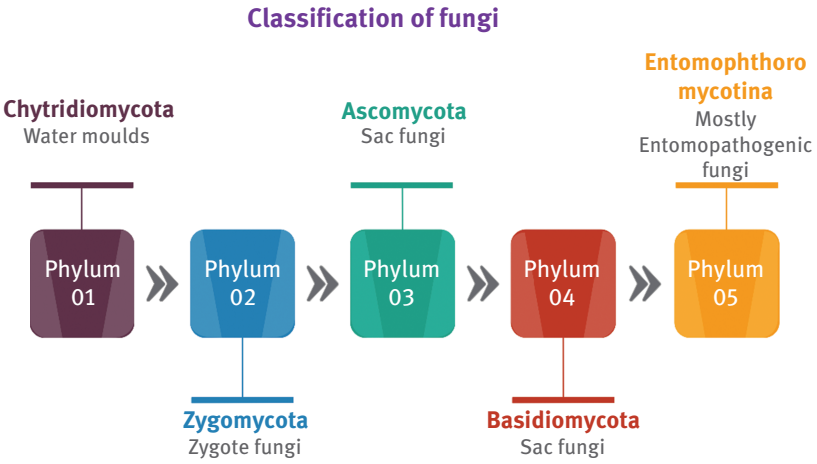

Figure 19.1: Classification of the fungal phylum.

19.2.1 Oomycota

Oomycota behave like a fungus because they have cellulose-based cell walls and plant-like biochemical properties. They are categorized as part of the kingdom Stramenophila rather than the kingdom Fungi [12]. Sexual reproduction can occur on the same or distinct hyphae between antheridia and archegonia. Lagenidiales and

Table 19.1: Classification of entomopathogenic fungi [6, 14, 19–37].

No.	Order	Family	Genus	Examples
Phylum: Chytridiomycota				
1.	Blastodadiales	Coelomomycetaceae	Coelomomyces	*Coelomomyces indicus*
2.	Chytridiales	Achlyogetonaceae	Myiophagus	*Myiophagus ucrainicus*
Phylum: Zygomycota				
1.	Mucorales	Mucoraceae	Mucor Rhizopus	*Mucor racemosus* *Rhizopus stolonifer*
Phylum: Ascomycota				
1.	Eurotiales	Trichocomaceae	Isaria	*Isaria farinosa* *Isaria fumosorosea*
2.	Onygenales	Ascosphaeraceae	Ascosphaera	*Ascosphaera apis* *Ascosphaera aggregate*
3.	Capnodiales	Capnodiaceae	Cladosporium	*Cladosporium cladosporioides*
4.	Hypocreales	Plectophaerelaceae	Verticillium	*Verticillium lecanii*
		Ophiocordycipitaceae	Hirsutella, Hymenostilbe, Tolypocladium	*Hirsutella citriformis* *Hymenostilbe dipterigena* *Tolypocladium cylindrosporum*
		Clavicipitaceae	Aschersonia, Metarhizium, Nomuraea, Nigelia	*Aschersonia aleyroids* *Metarhizium anisopliae* *Nomuraea rileyi* *Nigelia aurantiaca*
		Cordycipitaceae	Cordyceps, Beauveria, Gibellula	*Cordyceps lloydii* *Cordyceps militaris* *Beauveria brongniarti* *Beauveria bassiana* *Gibellula pulchra*
5.	Moniliales	Tuberculariaceae	Fusarium	*Fusarium coccophilum*

Table 19.1 (continued)

No.	Order	Family	Genus	Examples
Phylum: Basidiomycota				
1.	Septobasidiales	Septobasidiaceae	Septobasidium	*Septobasidium bogoriense*
Phylum: Entomophthoromycotina				
1.	Entomophthorales	Entomophthoraceae	Erynia, Entomophaga, Entomophthora, Batkoa, Furia, Massospora, Zoophthora	*Erynia aquatic* *Entomophaga grylli* *Entomophthora muscae* *Batkoa apiculate* *Furia americana* *Massospora cicadina* *Zoophthora radicans*
		Ancylistaceae	Conidiobolus, Pandora	*Conidiobolus coronatus* *Pandora blunckii*
		Neozygitaceae	Neozygites	*Neozygites floridana*

Saprolegniales contain entomophagous fungi. The genus Lagenidium consists of entomopathogenic fungi under the order Lagenidiales. The pathogen, *Lagenidium giganteum*, which affects mosquito larvae, has been investigated extensively [16, 17]. Crabs and aquatic crustaceans are also poisoned by several Lagenidium species [18]. These mosquito larvae are also infected by the Saproleginales species [19].

Domain: Eukarya
Kingdom: Fungi
Phylum: 5

19.2.2 Zygomycota

Zygomycota include multicellular, non-septate hyphae. The merging of gametangia forms zygospores. Molecular investigations have not revealed Zygomycota to be monophyletic [38]. EPF is categorized into two classes, Trichomycetes and Zygomycetes. Some trichomycetes EPF species can infect water insects such as mosquito larvae [39, 40]. Mucorales and Entomophthorales consist of EPF. Entomophthorales are major pathogens of both epigeal and soil-inhabiting insects. Around 200 species of entomogenous fungi have been reported in Entomophthorales [16], and Mucorales are a specific type of pathogens that infect only the weak insects.

19.2.3 Ascomycota

Ascomycota are septate haploid hyphae, along with yeasts. Sexual reproduction occurs by fusing modified hyphae or yeast-like cells, which leads to the formation of asci, which produce ascospores in groups of eight [41]. Cordyceps, the best-known ascomycete, contain over 300 insect-infecting species. The genus Ascosphaera has sexual dimorphism that causes chalkbrood disease in bees. The most common insect-infecting genera are *Beauveria, Metarhizium, Hirsutella, Paecilomyces, Aschersonia, Culicinomyces, Sorosporella*, *Lecanicillium*, and *Tolypocladium*. Biological or molecular investigations demonstrating the genetic link between teleomorphs and anamorphs can be utilized to confirm that these genera are related to one or more other genera [6, 42].

19.2.4 Basidiomycota

Basidiomycota includes fungi with dolipore septate hyphae as well as yeasts [41]. Hyphae generate sexual reproductive cells or basidia. Basidiospores are generated during nuclear fusion and meiosis on each basidium, commonly in groups of four [43]. Asexual reproduction is found apparently in a few species that produce conidia [41]. Probably, few Basidiomycetes have been discovered to be insect pathogens. The genera, Uredinella and Septobasidiales, have been described by certain scientists. Septobasidium is an infection-causing agent in insects, although it has a symbiotic connection with insects, namely, scales [40].

19.2.5 Chytridiomycota

Chytridiomycetes are categorized by their sexual reproduction system, including the unification of motile gametes and the synthesis of asexual, uniflagellate propagative spores [41]. EPF species are reported in Blastocladiales and Chytridiales. These are significant entomopathogens of aquatic insects. In Blastocladiales, the genera Coelomomyces is made up of more than 70 insect pathogenic species [44]. Coelomomyces cause infection to insects, including mosquitoes, black flies, midges, and backswimmers. In Chytridiales, the genus Myiophagus is pathogenically found on dipteran pupae with a specific affinity for insect-protective scale [40]. Various entomopathogenic fungi and their insect hosts are listed in Table 19.2.

Table 19.2: An overview of entomopathogenic fungal genera along with instances of insect hosts.

Fungus	Features	Target pest	References
Beauveria bassiana	Conidia are globous or broadly ellipsoid, ≤ 3.5 µm in diameter.	Coleoptera (Lepidoptera, Scarabaeidae, Castniidae, Curculionidae) Hymenoptera (Formicidae), Diptera (Tipulidae), Hemiptera (Lygaeidae, Cercopidae, Cicadellidae, Aleyrodidae, Aphididae, Pseudococcidae, Psyllidae) Lepidoptera (Noctuidae) Thysanoptera (Thripidae)	[41, 45]
Beauveria amorpha	Conidia is a curved or short cylinder with a flattened shape, and its dimensions are 3.5–5 × 1.5–2.0 µm.	Homoptera, Coleoptera, Hymenoptera, Lepidoptera	[46, 47]
Beauveria caledonica	Conidia is cylindrical or ellipsoidal and its size 3.7–5.2 × 1.9–2.3 µm	Coleoptera	
Beauveria asiatica	Conidia is oblong or ellipsoidal and its size 2.5–4 × 2 – 3 µm	Coleoptera: Cerambycidae Scarabaeidae	
Beauveria australis	Conidia is Sub-globose, moderately ellipsoid or ellipsoid, and globose are less common and its size 2–2.5 × 1.5–2.5 µm	Orthoptera: Acrididae	
Beauveria brongniartii	Conidia is ellipsoidal to sub-cylindrical and its size 2.5–4.5 µm.	Coleoptera: Scarabaeidae, Cerambycidae, European cockchafer, and other scarab beetle species	[48–49]
Beauveria kipukae	Conidia is globose or rarely ellipsoid, and its dimensions are 2–3.5 × 1.5–3 µm	Homoptera	[46, 47]
Beauveria malawiensis	Cylindrical and its dimensions 3.7–4.5 × 1.3–1.9 µm	Coleoptera: Scarabaeidae	
Beauveria sungii	Oblong or ellipsoidal and measures 2.5–3.5 × 1.5–2.5 µm	Coleoptera	
Beauveria varroae	Globose or broadly ellipsoid and measures 2–3.5 × 2–3 µm	Coleoptera: Curculionidae Acari: Varroidae	

Table 19.2 (continued)

Fungus	Features	Target pest	References
Beauveria lii	Cylindrical to ellipsoidal, occasionally obovoid, and its measures 3.1–10.1 × 1.4–3.6 µm	Coleoptera: Coccinellidae	[50]
Beauveria sinensis	Ellipsoidal to cylindrical and its measures 3–5 × 1.5–2 µm	Lepidoptera: Geometridae	[51]
Beauveria hoplocheli	Cylindrical to sub cylindrical and 3.5–4.5 × 1.5–2.5 µm	Coleoptera: Melolonthidae	
Beauveria rudraprayagi	Globose to sub-globose and its measures 2.5–4.0 × 2.5–4.0 µm	Skill worm	[55]
Metarhizium anisopliae	Conidia cells are cylinder and 9 µm length	Isoptera: Kalotermitidae, Rhinotermitidae, Termopsidae) Hemiptera: Aleyrodidae, Aphididae) Coleoptera: Curculionidae, Scarabaeidae), Hemiptera Blattodea: (Blattellidae, Blattidae), Thrips, fruit flies, mealybugs Orthoptera (Acrididae), Aphids, Sugarcane spittle bug	[45, 49, 53]
Metarhizium flavoviride	Conidia are ellipsoid greyish-green in masses, 7–11 µm in length.	Scarab larvae, Termites, Red-headed cockchafer	[45, 54–56]
Metarhizium chaiyaphumense	Perithecia are pyriform to oviform, and 320–380 µm wide, 550–670 µm long, Ascospores are hyaloid, filiform	Adult cicadas of the genus Platypleura.	[60]
Verticillium fusiporum	Fusoid conidia	Scale insects, aphids, and other insects	
Verticillium lecanii	Conidia ovoid to cylindrical with rounded apices 2–10 µm long and usually 1–1.7 µm wide.	Cabbage aphid, Mustard Aphid	[58]
Lecanicillium longisporum		Aphids	[45, 54–56]
Lecanicillium muscarium		Thrips and Whiteflies	
Lecanicillium lecanii		Whiteflies and thrips	[49]

Table 19.2 (continued)

Fungus	Features	Target pest	References
Isaria farinosa	Conidia are short fusoid to lemon-shaped ≤ 3 µm long, Synnemata present	Apple moth, Siberian pine caterpillar, and larch caterpillar	[59]
Isaria fumosorosea	Synnemata are usually smooth, uncolored long ovoid, with conidiophores and phialides, ≤ 4 µm rosy to tan to smoky pink in mas	Whiteflies, aphids, thrips, psyllids, mealybugs, and fungus gnats	
Isaria lilacinus	Conidia are fusoid to ellipsoid and 2–3 µm long	Wax moth	[57]
Hirsutella thompsonii	Conidia globose with a wrinkled or smooth but no visible slime layer	Mites, Acari	[45, 54–56]
Hirsutella citriformis	Synnemata are usually long, with numerous browns or grey with many short lateral branches.	Leaf and planthoppers, Hemiptera	[57]
Hirsutella rhosiliensis	Conidiophores cells are conidia with short, narrow neck and orange segments straight on one side and curved on the other or ellipsoid. Not forming synnemata	Mites	
Cordyceps militaris	Stromata with a swollen fertile section at the tip, < 10 cm high, densely clavate, and unbranched orange.	Lepidopterans	
Cordyceps lloydii	Stromata, white to cream-colored, ≤ 1 cm tall, with the discoid apical fertile region and slightly immersed perithecia.	Ants	
Cordyceps mrciensis	Stromata black to dark brown, < 8 cm tall with an elongated and slightly enlarged fertile part.	Spiders	
Cordyceps tuberculate	Perithecia are slightly saturated sulfur to intense yellow and dispersed towards apices, Stromata off-white	Lepidoptera	

Table 19.2 (continued)

Fungus	Features	Target pest	References
Cordyceps takaomontana	Fruit bodies are tubular or clavate, pale yellow, and shaped from pseudo sclerotium and 1–5 cm long, diameter up to 3.5 mm	Lepidoptera	
Entomophaga aulicae	Conidiogenous cells are non-elongated with narrow-necked subtending conidia.	Lepidoptera	
Entomophaga maimaiga	Conidia are obclavate or pyriform, hyaline, 16–28 µm width, and 20–36 µm length.	Gypsy moths	
Entomophaga calopteni	Formation of resting spores but no primary conidia	Melanopline (grasshopper)	
Entomophaga grylli	Conidiophores and conidia are formed by hyphae having cell walls.	Diverse acridids	
Entomophthora culicis	Binucleate Conidia	Black flies and mosquitoes	
Entomophthora muscae	Primary conidia and minute secondary conidia are present.	Muscoid flies	
Entomophthora planchoniana	Primary conidia are bell-shaped plurinucleate with a sharp-pointed tip and flattened papilla. Conidia dimensions are 15–20 to 12–16 µm.	Aphids	
Erynia aquatic	Conidia is clavate and measuring 30–40 × 15–18 µm	Diptera	
E. rhizospora	Conidia are straight to lunate and measure 30–40 × 8–10 µm in length	Trichoptera	
E. conica	Conidia are curved, tapering to a sub-acute apex and measuring 30–80 × 12–15 µm in length.	Diptera	
E. ovispora	Conidia are ovoid to ellipsoid and measures 23–30 × 12–14 µm.	Nematoceran dipterans	

Table 19.2 (continued)

Fungus	Features	Target pest	References
Cladosporium cladosporioides	Conidiophores brownish color, irregularly branched, 40–350 µm long 2–6 µm wide.	Aphids	
Septobasidium bogoriense	Basidiomata are branches and measure between 1–5 cm wide, 2–15 cm long, whitish-grey to greyish-brown	Scale insects	
Batkoa apiculata	Papilla often with pointed exterior and Conidia 30–40 µm diameter.	Homopterans and flies	
Tolypocladium cylindrosporum	Conidia are cylindrical or straight, or slightly curved.	Small Dipterans	
Ascosphaera aggregate	Ascospores 4–7 µm long ovoid to cylindrical.	Leaf-cutting bees	
Fusarium coccophilum	Macroconidia with transverse septa	Scale and other insects	
Aschersonia aleyroids	Stroma 2 × 2 mm tall. Thin halo hyphae spread throughout the leaf surface, ranging from orange to pink to cream in color.	Coccicds & aleyroidids	
Gibellula pulchra	Synnemata orange-yellow, Conidiophores are bulging from the surface of white lilac	Spiders	
Hymenostilbe furcate	Creamy white synnemata, conidiogenous cells two to seven forked denticles	Hemipteran insects,	
Nomuraea rileyi	Conidia are ovoid, Conidial mass grey-green covering a host	Lepidoptera	
Paecilomyces fumosoroseus		Mustard aphids, Diamondback moth	[45, 54–56]
Peacilomyces lilcinus		Mustard Aphids	

Table 19.2 (continued)

Fungus	Features	Target pest	References
Nigelia aurantiaca	Stromata up righted was cylindrical to club-shaped, yellowish or brownish. Perithecia submerged, wobbly, or closely composed, 320–440 µm wide, 520–680 µm long.	Lepidoptera	[57]
Conidiobolus thromboides	Conidia is pyriform, papilla emerges into spore, No capilliconidida or microconidia.	Aphids/Thrips/ Whiteflies	
Furia Americana	Conidia is obovoid and measures 28–35 × 14–16 µm.	Cyclorrhaphan muscoid flies	
Massospora cicadina	Conidia 1–6 nucleate	17-year Cicada	
Neozygites floridana	Zygospores sub globose dark brown, and Conidia are 10–14 µm diameter	Tetranychid mites	
Pandora blunckii	Conidia pyriform and measuring 15–20 × 7–11 µm	Lepidoptera	
Pandora dephacis	Conidia is clavate and measures 30–35 × 12–18 µm.	Hemiptera	
Zoophthora phytonomi	Conidia cylindrical, resting spores colorless	Coleoptera on alfalfa	
Coelomomyces indicus	Sporangia are anastomosing and measuring 25–65 × 30–40 µm	Mosquitoes	
Myiophagus ucrainicus	Zoospores are rarely present. Sporangia is 20–30 µm diameter and golden brown globose reticulated surface.	Beetle larvae, Scale insects, Mealybugs, Weevils, and Lepidoptera.	

19.3 Mode of action

Insect-pathogenic fungi must overcome various host challenges to produce sufficient new infectious spores in every generation to maintain a healthy population. Unlike other fungi, entomopathogens may infect their hosts directly through the exoskeleton or cuticle, where the non-feeding stages, such as eggs and pupae, can also be affected. High humidity encourages germination; so invasion occurs more readily between the mouthparts and the intersegmental folds and through the cuticle, when

unsclerotized [60], [61]. The cuticle is the first point of contact between the fungus and insect. The insect dies through various causes due to this invasion, including tissue injury, nutritional loss, mycoses, and bodily toxins. Entomopathogenic fungi have evolved host surface attachment and recognition mechanisms. The adaptive reactions in EPF include the production of infectious structures such as appressoria or penetration tubes, secondary metabolites, hydrolytic, assimilatory, and detoxifying enzymes. Various steps involved in EPF infection are depicted in Figure 19.2.

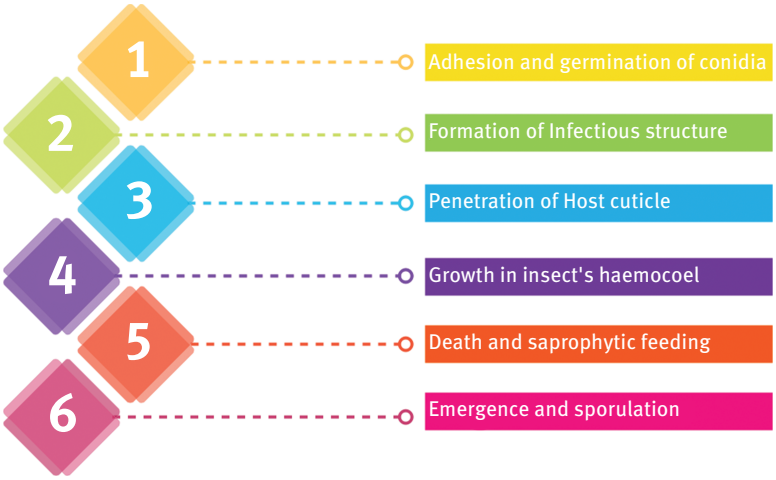

Figure 19.2: Steps involved in the infection process of entomopathogenic fungi.

On the other hand, insects have evolved several defense mechanisms against illnesses, including creating epicuticular antimicrobial lipids, proteins, and metabolites, cuticle shedding during growth, induced fever, burrowing, and grooming, which are examples of biochemical-environmental adaptations. Various defense mechanisms of insects against EPF are shown in Figure 19.3. These features help insects prevent infections from penetrating the cuticle [65]. There is a coevolutionary arms race between pathogens and the target insects. Current research implies that the cuticular surface plays a role in the pathogen-host coevolutionary arms race. Surface interactions cause the infection to produce Mycosis or the host to protect itself [63]. The life cycle of entomopathogenic fungi in insects is shown in Figure 19.4.

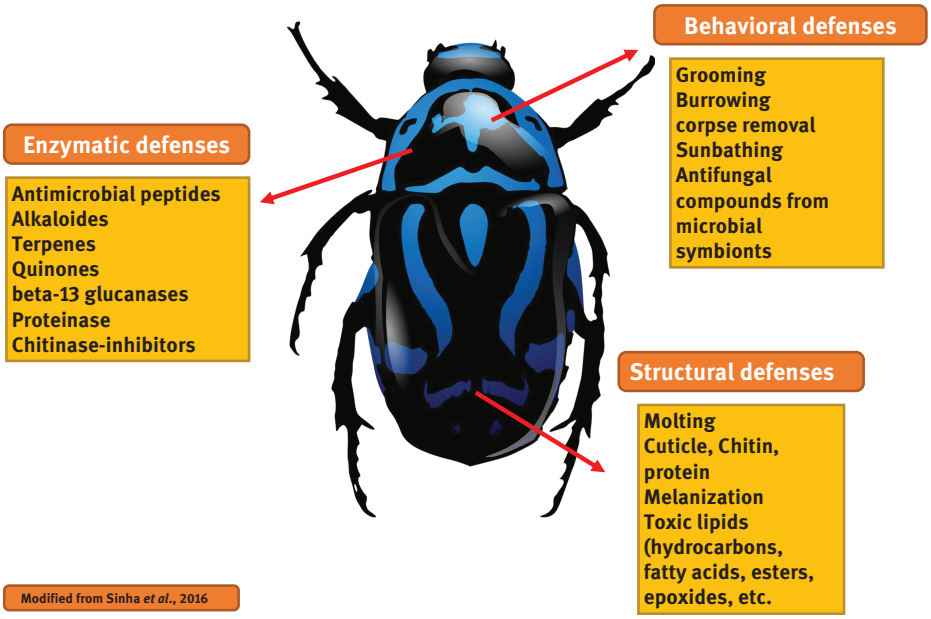

Figure 19.3: Defense mechanisms in insects against entomopathogens.

19.3.1 Adhesion and germination of conidia

Wind or water might aid attachment, which is a passive technique. Mycosis begins with spores (conidia or blastospores) attaching to the cuticle surface of a susceptible host. However, certain insects have preferred spots to enter the host's cuticle [64]. Insect cuticle are complex structures that change their composition with time. Epicuticle is the cuticle's outermost layer, followed by the procuticle, which is further divided into Exo, meso, and endo-cuticular layers. Finally, the epidermis surrounds the inner structures and is the innermost layer.

A layer of interwoven fascicles of hydrophobic rodlets made up of protein hydrophobins was identified in the dry spores of *B. bassiana* [65]. This rodlet layer is only seen in conidial cells and not vegetative cells. Rodlets exert non-specific hydrophobic forces on the cuticle, causing dry spore adhesion [66]. In *B. bassiana*, two hydrophobins (Hyd1 and Hyd2) influence the rodlet layer formation, contributing to adhesion to hydrophobic surfaces, pathogenicity, and cell-surface hydrophobicity [65, 67]. *M. anisopliae* has Mad1 and Mad2 adhesion genes [68]. Mad1 deficiency reduced cuticle adhesion, germination, blastospore production, and pathogenicity.

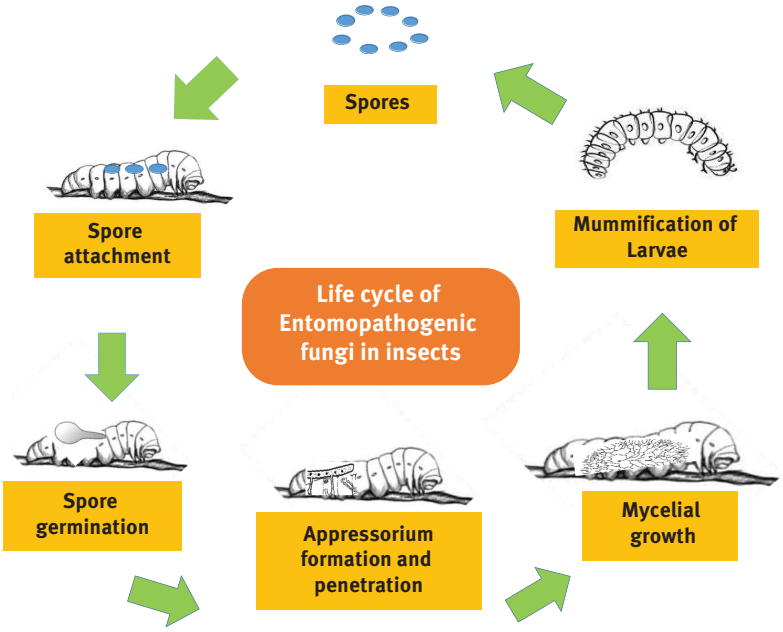

Figure 19.4: Life cycle of entomopathogenic fungi in insects.

The non-specific (passive) adsorption stage is attributed to hydrophobins, whereas the target-specific (active) stage is attributed to Mad2 adhesions [63]. Once a pathogen penetrates and attaches to the host cuticle, it germinates, and the availability of nutrients, oxygen, water, pH, temperature, and the impact of toxic host surface chemicals all play a role in its continuing growth. Fungi, with extensive host ranges, germinate in cultures in response to a wide range of non-specific nitrogen and carbon sources. [69] On the other hand, entomopathogenic fungi with a limited host range appear to have particular germination demands. For instance, *B. bassiana* has been proven to thrive on insect hydrocarbons, including methyl-branched and aliphatic alkanes [70].

19.3.2 Formation of infectious structure

The fungus infects the host by penetrating the cuticle. The epicuticle (outer cuticle) lacks chitin but it is structurally complex. It includes phenol-stabilizing proteins and coated by a waxy coating of fatty acids, lipids, and sterols [71]. The procuticle (inner cuticle) contains most of the cuticle's components. A protein matrix holds chitin fibrils, lipids, and quinones. For the fungus to penetrate the cuticle, it has to form a germ tube or an appressorium [71]. This appressorium formation focuses its physical and chemical energy across a narrow region and ensures efficient entry.

The host's surface topography and intracellular second messengers, such as Ca2 + and cAMP, impact appressorium formation [65].

19.3.3 Penetration of host cuticle

Entomopathogenic fungi penetrates the insect body through the cuticle to feed on nutrition. Fungal invasion requires both mechanical and enzymatic pressure. The site in the epicuticle appears as a black, melanotic lesion after penetration [72]. Proteins are an insect's recyclable resource and an important cuticle component. Proteases, lipases, and chitinase degrade the cuticle during the entomopathogenic fungi penetration. Proteases include trypsin, chymotrypsin, esterase, collagenase, and chymoelastase [73]; [74]. Endoproteases (PR1 and PR2) and aminopeptidase are the first cuticle enzymes that are generated and generally associated with appressoria formation [75]. Active endoprotease in the fungi helps to penetrate the cuticle, as protein constitutes up to 70% of the insect cuticle.

Since the insect cuticle is complete and is among the enzymes involved in the cuticle penetration, N-acetyl glucosaminidase is produced more slowly than proteolytic enzymes. Hence, it requires the coordinated activity of several enzymes to penetrate the cuticle. In *B. bassiana*, cuticular lipid breakdown involves eight CYP genes, four catalases, three lipases/esterase, long-chain alcohol and aldehyde dehydrogenases, and a putative hydrocarbon transporter protein [76,77]. Temperature, humidity, and light also affect insect cuticle adherence, germination, development, and penetration. Insect hemocoel is immediately penetrated by the fungus via the cuticle, employing extracellular enzymes (chitinases, lipases, esterase, and numerous proteases), and many enzymes work together to penetrate the cuticle. The fungus then disperses into the hemolymph through blastospores or a yeast-like structure, reaching the insect's respiratory system for optimal feeding. Finally, the insect dies via a combination of causes, including mechanical harm from tissue invasion, nutrition loss, and toxin formation (toxicosis) [62].

19.3.4 Production of toxins

Entomopathogenic fungi produce major toxins such as destruxins, efrapetins, beauvericin, leucinostatines, and bassianolide. Various toxins produced by entomopathogenic fungi are described in Figure 19.5.

Deuteromycetes pathogens create a variety of fungal toxins associated with insect health. The effects of these toxins on diverse insect tissues have been shown (Table 19.1). Cytotoxins may be involved in the breakdown of cells prior to hyphae entry. Neuromuscular toxins cause paralysis, sluggishness, and reduced excitability in insects infected with fungi, according to [78]. *B.bassiana*

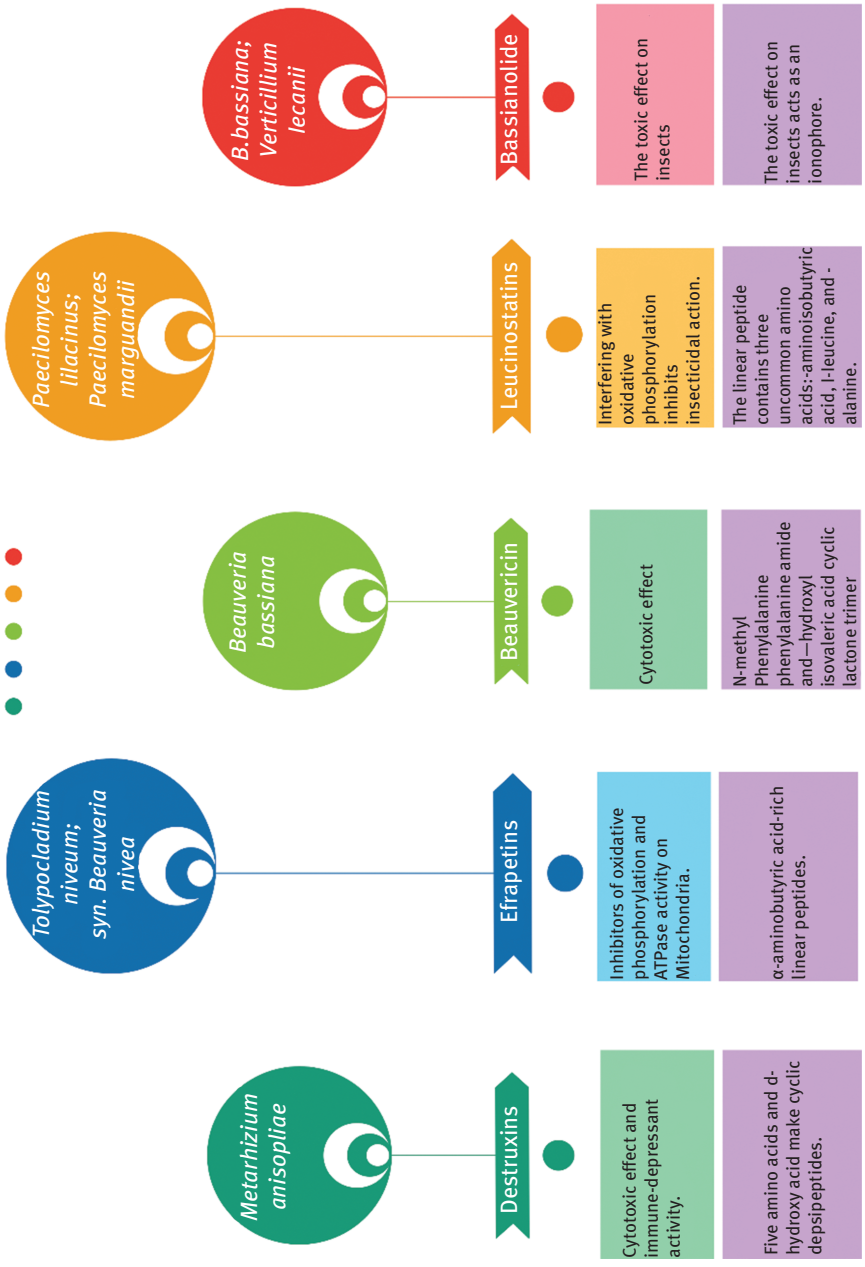

Figure 19.5: Toxins produced by entomopathogenic fungi [62].

toxins include beauvericin, beauverolides, bassianolide, and isarolides [79]. *Metarhizium anisopliae* infestation forces insects to produce destruxins and cytochalasins. DTX depolarizes lepidopteran muscle membranes and changes hemocyte function by activating calcium channels [80].

19.3.4.1 Destruxins

Destruxins are cyclic depsipeptides that comprise five amino acids and a D-hydroxyl acid. It was first found in *M.anisopliae* [81–83]. They are produced after the mycelial growth and suppress the insect's immune system. In addition, Destruxins may help the pathogen to establish itself in the host. These poisons kill insects after a fungus infestation [84].

19.3.4.2 Efrapeptins

Soil hyphomycetes, *Tolypocladium niveum* and *Beauveria nivea*, produce efrapeptins, a complex peptide antibiotic. Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera) is poisoned by efrapeptins. All peptides are strong mitochondrial oxidative phosphorylators and ATPase inhibitors when tested against entomopathogenic fungi (*M.anisopliae* and *T.niveum*) preparations. These peptides are presumably catalytic-site competitive inhibitors that bind to the soluble F′ component of the mitochondrial ATPase [85].

19.3.4.3 Bassianolide

Bassionolide is an ionophore of divalent cation. It is cytotoxic and insecticidal against mosquito larvae, blowflies, and the Colorado potato beetle. It has been found in *B. bassiana*, *P. fumosoroseus*, *Fusarium semitectum*, *Fusarium moniliforme* var. *subglutinans*, and *Polyporus sulphureus*, a plant pathogenic basidiomycetous fungi [62].

19.4 Alternate modes of action by entomopathogenic fungi

Insect infection by microbial ingestion is typical when the entomopathogen is a virus, bacterium, or protozoa; however, it has been reported that entomopathogenic fungi can utilize oral and respiratory routes as an alternative to cuticle penetration

for their entry [88–91]. These strategies may provide an opportunity to boost efficiency against fungal-resistant arthropods by embedding antifungal compounds in their cuticle [92], [76], [93]. The first reports on entomopathogenic fungus infection pathways were published [94–96]. After several years, with no new understanding, researchers began to look into the molecular processes behind these infection pathways. Next-generation technology enabled the collection of vast volumes of genomic and transcriptome data from fungus and arthropods [90]. Alternative routes of entry of entomopathogenic fungus into the host are depicted in Figure 19.6.

19.4.1 Oral infection route by entomopathogenic fungi in terrestrial insects

Researchers have long been intrigued by the idea of entomopathogenic fungi invading the oral/gastrointestinal tract; however, much remains unknown. A century ago, scientists advocated that *B.bassiana* infect pine weevils' through the oral cavity [96]. From the mid-1940s to the mid-1980s, a discontinued study series found *M. anisopliae* hyphae surrounding the implantation of the mandibles and oesophagus of *Ephestia kuhniella* Zeller (Lepidoptera: Pyralidae) and non-germinated *M. anisopliae* spores in the stomach of *Oryctes* larvae. Scientists concluded that oral infection was frequent on maxillary palps and the head of *Schistocerca gregaria* by feeding on leaves infected with *M. anisopliae* spores [95]. Some researchers reported substantial mortality and hyphal development in all regions of the digestive tube and no apparent evidence of germination in the stomach, suggesting fungal conidia invade through the beetle mouthparts [88].

More evidence that fungal conidia adhere to, germinate on, and enter through the buccal apparatus to kill insects, rather than penetrating the gut that is protected by its microbiota, comes from *S. gregaria* fed *M. anisopliae* conidia [78]. In recent research on *Sitophilus granarius* Linnaeus (Coleoptera: Curculionidae), it was found that conidia of *B. bassiana* and *M. anisopliae* could infect the insects' digestive tube cuticles. They were disinfected to prevent cuticular breaching and killing of the beetle. However, this study found inadequate histological data to support the stated idea [91].

According to research on terrestrial insects, the mechanisms by which they swallowed spores and kill the host remain unknown. Because conidia do not germinate in the stomach, it appears that fungal spores preferentially adhere to the buccal cavity rather than the digestive system. Furthermore, studies are needed to better understand the physiological and molecular changes that occur when entomopathogenic fungus spores are eaten [87].

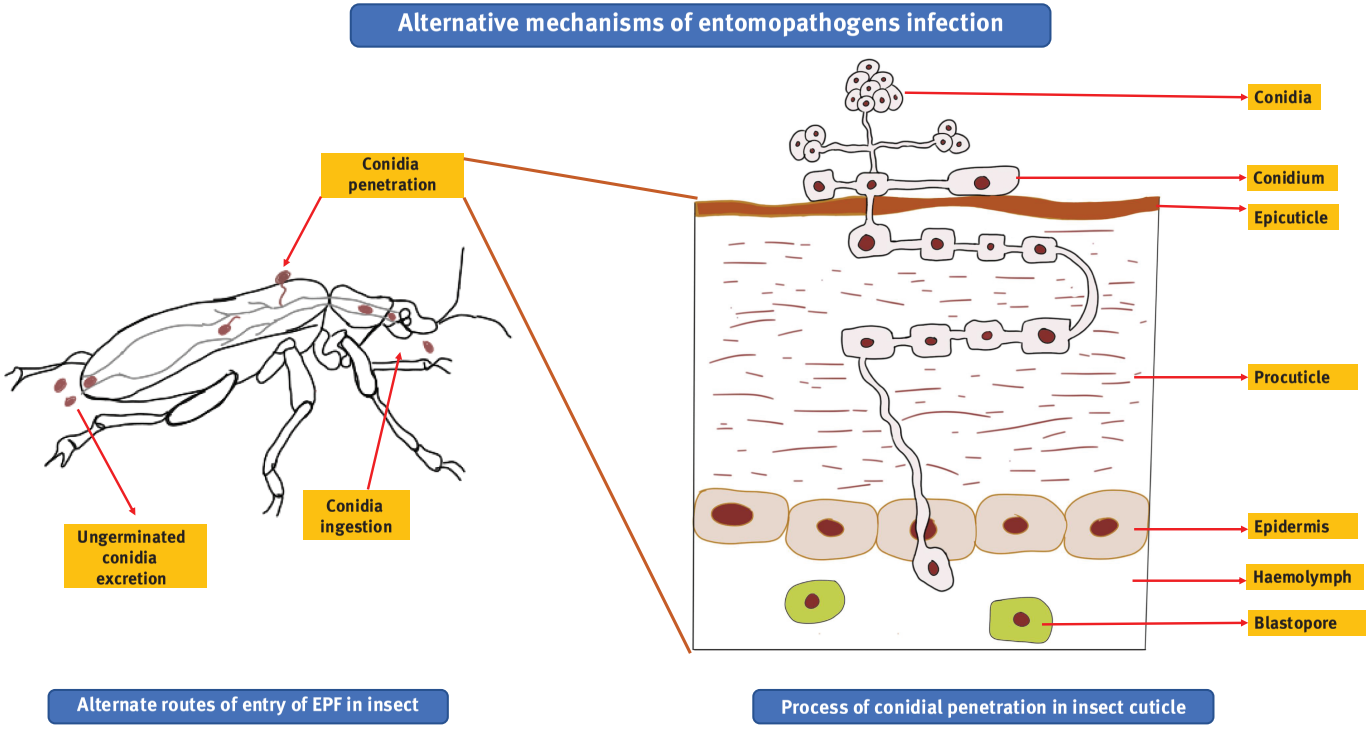

Figure 19.6: Alternative routes of entry of entomopathogenic fungus into the host.

19.4.2 Oral infection route by entomopathogenic fungi in aquatic insects

Infection by entomopathogenic fungi that have adapted to terrestrial hosts appears to be possible in mosquito larvae. While entomopathogenic fungi have pathogenic traits that kill hosts, they do not infect as actual aquatic fungal infections [97]. Conidia of *M. anisopliae* have a hydrophobic surface and float. Conidia connect to the siphon tip when the larvae open their perispiracular valves for air intake; hyphae develop into the trachea and suffocate the insect. Lesser conidia can be given to larvae after treating a non-ionic deterrent. Conidia live in insect's guts and kill them with poisons, but they do not infect the rest of the animal [94].

Because conidia are indigestible for larvae and occupy the digestive tract, nutritional support is essential in this last entry route [98]. Ingestion of conidia happens; however, how the infection develops beyond the entrance point differs, depending on the host and pathogen. However, *M. anisopliae* conidia in the gut of *S.gregaria* showed fungitoxicity, which is dependent on the gut flora [78, 99]. The head and anal areas were the most common infection sites for *Aedes aegypti* Linnaeus (Diptera: Culicidae) when administered with *B. bassiana* conidia [100]. Insect mortality appears to be connected with autolysis that is induced by caspases, which are protease enzymes implicated in apoptotic processes. Some scientists reported that the infected larvae's Hsp70 modulates the protease inhibitor mechanism [101]. Aquatic larvae of culicid dipterans have anal papillae that adhere to the anal papillae, rather than the cuticle. This fungus may infect the host through the intestinal cavity, but does not adhere to the cuticle [102], [103]. Few researchers tested the insecticidal activity of *Culicinomyces clavisporus* (Hypocreales: Cordycipitaceae) isolates on *Aedes aegypti* larvae, eggs, and adults[104]. They observed that they killed faster at a lower dose after several serial repassages of *Aedes aegypti* larvae [105].

19.4.3 Molecular evidence for oral infection

Researchers revealed that some entomopathogenic fungi contain a repertoire of genes that allow them to have oral toxicity. For example, *B. bassiana* has at least 13 heat-labile bacteria-like enterotoxins, whereas *M. robertsii* has six. Additionally, *B. bassiana* has eight Cry-like delta enterotoxins and three bacteria-like zeta toxins proteins; the rest of the entomopathogenic fungi have one [67, 106, 107]. Various genes that are involved in the oral infection of Entomopathogenic fungi are mentioned in Table 19.3.

Table 19.3: Genes of entomopathogenic fungi involved in the oral infection [106].

Gene family	No. of genes in EPF		Description of genes
	Metarhizium anisopliae	*Beauveria bassiana*	
Zeta toxins, bacterial-like	0	3	Bacterial heat-labile enterotoxin IIB, A chain (enzymatic), and IIA A
Cry-like delta enterotoxins	0	8	Bacterial delta endotoxin, N-terminal
Heat labile bacterial-like Toxins	6	13	Bacterial toxin

19.5 Mass production

A large load of inoculum is required to manage insect pests at the field level. It is not feasible in natural settings because the inoculum is less in fields. So, the EPF should be manufactured in large quantities in vitro and applied in the field to control insect pests. The success of deploying entomopathogenic fungi in pest control is dependent on reliable and cheap methods for mass production.

19.5.1 Methods of mass production

Different fungi have diverged nutrient requirements. The host range of fungi that belong to the class oomycetes, zygomycetes, and chytridiomycetes is very narrow and are specific in their nutrient specificity. On the other hand, fungi that belong to the class ascomycetes enjoy an extensive host range so that the fungi can be grown in various substrates; a complex mixture of nutrients is best suited. The major concern is to mass-produce entomopathogenic fungi cheaply without compromising their efficacy [108].

19.5.1.1 Solid substrate fermentation

This is the primary production method with no free moisture, which simulates the natural conditions and results in aerial conidia production by the fungi [109, 110]. The other name, the solid-state fermentation, is used interchangeably. Most commonly used media include rice, wheat bran, cracked barley, millets, corn, rye, sorghum, and peat soil [62, 109]. Sugarcane bagasse has scope to be used as a supporting matrix to

boost growth and sporulation [111]. The media should be moistened to the optimum level to support fungal growth. Take the moistened media in an autoclavable polyethylene cover and autoclave at 121 °C at 15 psi for 15–20 min. Inoculate the media with seedling inoculum prepared in an agar plate. Transfer it to an Erlenmeyer flask containing 100 mL liquid and it serves as the starting material for solid substrate fermentation. The fungi-inoculated polyethylene bag should be incubated at 25 °C and 12 h light: 12 h dark photoperiod for 10–14 days. Then, dry the culture media until the moisture level comes down to 5% [62, 112]. This method is labor-intensive, requires a long incubation period, and may involve inevitable contaminations. [110].

19.5.1.2 Liquid fermentation

Liquid fermentation is of two types, namely, submerged fermentation and stationary liquid fermentation. In submerged fermentation, continuous agitation and aeration are provided to the media, favoring blastospore formation, micro-cycle conidia, and microsclerotia. Submerged fermentation is a commonly deployed method to mass-produce fungi such as *Beauveria, Metarhizium, Isaria,* and *Lecanicillium*. However, Conidia is not possible in this method for obtaining 100% blastospores or unblended micro-cycle. The second method is stationary liquid fermentation, in which fungi grow and conidiate on the standstill liquid media, in which mycelia and conidia are formed. In large-scale production, such as in industries, the fermentation is accomplished in a fermenter [109]. This method has the added advantages such as automation of the process and scaling up [110].

19.5.1.3 Biphasic system

The seedling inoculum is prepared by liquid fermentation in the biphasic system, which is the starting material for solid fermentation. Therefore, this method is also referred to as liquid-solid fermentation. Using blastospores produced from liquid fermentation as inoculum for the solid substrate, fermentation yields higher conidia than conidia as inoculum [111].

19.5.2 Formulations of EPF

EPF Formulation is a blend of viable conidia meant for successful pest control. Formulating EPF prolongs the shelf life, handling, application, storage, safety, and effectiveness. EPFs come in many different formulations. Formulations are of two types, i.e., solid and liquid formulation. Formulations are usually described using abbreviations [113].

19.5.2.1 Dust

The active ingredient is mixed with finely ground solid inert material such as clay, talc, etc. The particle size of dust formulation ranges from 50–100 micrometers. The dust formulation is then applied directly to the target insect. The choice of inert material is determined by its characteristics such as anticaking, UV protectant, and adhesiveness. Usually, the titter of microorganisms will be 10% [114].

19.5.2.2 Granular

A ready-to-use free-flowing solid formulation with a predetermined granule size range is available[115]. Formulations have larger and heavier particles with sizes ranging from 100–1000 micrometers for granules; micro granules range from 100–600 micrometers in size. The inert materials used in these formulations include kaolin, silica, polymers, starch, attapulgite, ground plant residues, and dry fertilizers. The concentration of an *a.i.* is 5–20%. The granular formulation is mostly applied to the soil for controlling nematodes, weeds, and soil-dwelling insects. The *a.i.* is released slowly from the granules. A specific soil moisture level is required while using granular formulation to act effectively [114].

19.5.2.3 Wettable powder (WP)

A powder formulation is applied as a suspension after dispersion in water [115]. It is free from moisture. The active ingredients are mixed with adjuvants such as a surfactant, dispersing agent, wetting agents, or inert material, crushed to a fine powder with a particle size of 5 microns, and applied as a suspension by mixing with water. Extended storage stability, efficient water dispersal, and ease of application with standard sprayers are just a few of the benefits [114].

19.5.2.4 Water dispersible granule (WDG)

Same as wettable powder, this formulation is developed to overcome the dustiness problem in wettable powder [114].

19.5.2.5 Emulsion

The emulsion contains an immiscible liquid in which droplets of size 0.1–1 micrometer are dispersed. It is of two types, namely, normal emulsion (oil in water) and invert emulsion (water in oil). However, it should be combined with water [114].

19.5.2.5 Suspension concentrate (SC)

A steady suspension of active ingredients with water is to be diluted before usage [12]. These are solid active substances that have been extensively crushed and disseminated in water. Agitation is required before application to keep the particles evenly distributed. Many ingredients are used in SC formulations, including wetting agents, dispersion agents, foam inhibitors, gelling agents, and so on. They are created using a wet grinding process, with particle sizes ranging from 1 to 10 μm.

19.5.2.6 Oil Dispersion

Solid active ingredient is dispersed in a liquid other than water, most often in oil; however, plant oil is predominantly used. This formulation has excellent penetration and spreading activity and is also very helpful in delivering water-sensitive ingredients [114].

19.5.2.7 Aerosol

One or other active ingredient is mixed with a solvent. Mostly, aerosol formulation contains a lower percentage of active ingredients. Aerosols are delivered as fine droplets. They are employed in greenhouses, indoors, and in localized outdoor areas [116].

19.6 Methods of application

Several factors are to be considered before applying EPF. They include spore concentration and environmental conditions. Currently, four common methods are plant or root dipping, foliar application, soil application, and vector transmission. Foliar spray is a promising and convenient application method among all those methods. Root dipping can be followed in crops that are transplanted. Soil application of EPF is used for targeting soil-dwelling insects. Major entomopathogenic fungi formulations and their host along with trade names are provided in Table 19.4.

Table 19.4: Popular entomopathogenic fungi, host and their commercial names.

Fungi	Host	Commercial name
Lagenidium giganteum	Mosquito	Lagenex
Coelomyces psorophorae	Mosquito	Lagenex
Conidiobolus Coronatus	Aphids, flies, caterpillars	–
Pandora	BPH and GLH of Rice	–
Beauveria bassiana	Cotton bollworms, coffee berry borer, DBM	Beveroz, Sun bio Bevigaurd, Dr. Bacto's Brave
Metarhizium anisopliae	Sugarcane pyrilla, Rhinoceros beetle	Metarhoz, Almid, Dr. Bacto's Meta, Sun Bio Meta
Lecanicillium lecanii	Whiteflies, scales, aphids, mealybugs, planthopper, thrips	Biogreen, Vertilac, Biocatch, Verticare
Isaria fumosorocea	Whitefly	Priority
Hirsutella thompsonii	Phytophagous mites	No mite

19.7 Approaches toward the application of entomopathogenic fungi

EPF is a natural enemy of insect pests and regulates their ecosystem population. In recent years, EPF-based biological control programs followed the same inundative spraying pattern and inoculative biological control strategy [117]. In addition, entomopathogenic fungi have several properties that make them an excellent alternative or adjunct to synthetic pesticides [118]. The main approaches in the application of EPF for pest management are described in Figure 19.7. They are classical control/introduction, augmentation, and conservation.

19.7.1 Classical control

In this approach, natural enemies are used to control exotic hosts that become invasive in new environments/localities where those specific natural enemies are absent [119]. So the correct identification of pests and their locality is necessary for a

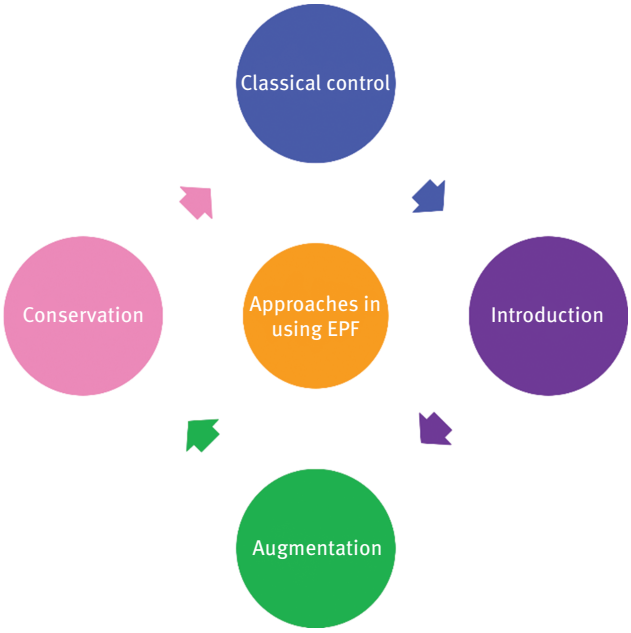

Figure 19.7: Approaches toward the application of entomopathogenic fungi.

successful classical biological control program. The early history of the application of EPF using this approach starts with the introduction of *Entamophaga maimaiga* (Entamophthorales: Entamophthoraceae) that was isolated in Japan and used in the US for the control of larvae of the Gypsy moth, *Lymantria dispar (shah)*. Subsequently, a successful permanent introduction of EPF was reported owing to the conidial movement of fungi and human manipulation [120]. Following this, another entomopathogenic *Zoophthora radicans (*Zygomycetes: Entamophthothorales) from Israel had been successfully introduced in Australia for the management of spotted alfalfa aphid *Therioaphis trifolii* (Homoptera: Drepanosiphidae) [3]. At the same time, standards for collection and importation/exportation of entomopathogen should be framed by IPPC.

19.7.2 Augmentation

In augmentation, control is brought about by manipulating within organisms. This technique is carried out by a mass production technology and genetic improvement to promote efficiency and population [5]. In the case of fungi, augmentation usually involves applying in vitro-produced conidia or mycelia in aqueous suspensions in a greenhouse or field, often along with formulations to improve its persistence and

infectivity [6]. Hypocreales are more generalized, while entomopathoromycota are more target-specific; both have little influence on natural enemies [118].

19.7.3 Conservation

In the case of conservation, manipulation is carried out in the environment. In this technique, the habitat is modified to favor an organism's potentiality, virulence, and the successful establishment of epizootics. Conservation should be the first consideration in biocontrol programs [5]. Usually, entomopathogenic fungi have two phases in their life cycle: a typical mycelia development phase that occurs primarily well outside the host body and a budding phase that occurs mainly in the hemocoel of the insect host [122]. Enhancement or conservation can be done by

- Maintaining the hosts and diversity in that ecosystem to overcome the unsuitable situation of the biocontrol agent
- Manipulating alternate host
- Manipulating suitable environmental conditions such as temperature and humidity while building a greenhouse/polyhouse/glasshouse [121]
- Infectivity of *B. bassiana* in the soil can be improved by radiation of the modified hyphal strands [123]

19.7.4 Persistence of entomopathogenic fungi

Fungi require specific circumstances that favor the growth, development, and persistence of pathogens to control insect pests effectively. According to many studies, the change in the organism's reaction in laboratory and field conditions is the reason for the non-persistence of many fungal entomopathogens, and hence stabilizers should be used. When there is a lack of a host and a non-favorable environmental condition sustains, most entomopathogenic fungi produce either meiotic resting spore (zygospore) or mitotic resting spore (azygospore) and persists in the soil until a favorable time arises [49, 124].

19.7.5 Safety

There is fear that using entomopathogenic Hyphomycetes as biocontrol agents might be dangerous to the applicator or the environment, just like other pathogens. Despite their facultative character and rather extensive host ranges, entomopathogenic Hyphomycetes appear to provide negligible harm to humans, domesticated pets, fauna, and the ecosystem [125]. A no-risk situation cannot be assured for chemical pesticides and biopesticides [126, 127]. As *B. bassiana* and *B. brangroftii*

are soil-dwelling organisms, they show effects in soil pests and soil-inhabiting non-target species [127]. Field observations did not show any possible adverse effects on bees, natural enemies, and earthworms. In contrast, laboratory studies negatively affected the carabids, cicindellids, and collembolans when pushed to stress conditions [125, 127]. With respect to the effects of *Beauveria* species on mammals and humans, there are no pathogenic, allergic, or toxic hazards. [125, 126, 128–131]. A report documented that a person under immunosuppressive therapy was infected with *B. bassiana* in deep tissue [132]. *B. brangroftii* showed non-pathogenicity over warm-blooded animals and not even a single negative report over decades of use [133, 134]. Investigations into the behavior of a transgenic *M. anisopliae* ARSEF 1080 strain in the soil under field conditions revealed that the transgenic strain did not suppress the culturable native fungal microbiota [135]. *M. anisopliae* was first experimented with inhalation on mice, guinea pigs, and rats, resulting in no evidence of allergy [136–138]. An isolate of *M. anisopliae* var *acridium* showed severe dermal allergic response in humans [131]. Studies beyond allergic reactions on humans, such as carcinogenic, serological, and more genetic studies, have also been commenced [139]. Mitosporic fungi are typically safe and nontoxic, with no or mild mammalian toxicity and persistent toxicity [140]. Various advantages and disadvantages in using entomopathogenic fungi as a biocontrol agent are described in Figure 19.8.

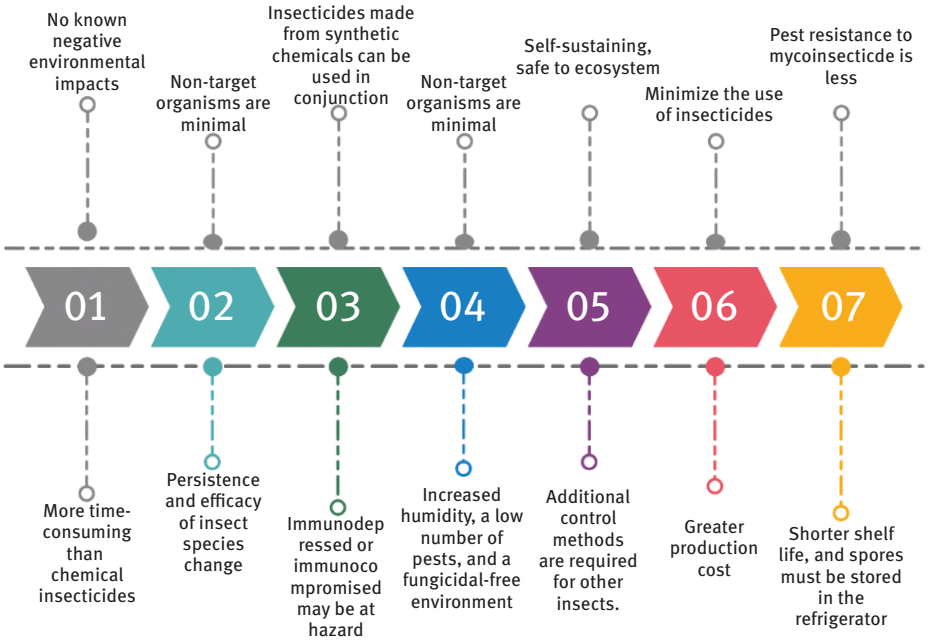

Figure 19.8: Advantages and disadvantages of using entomopathogenic fungi as a biocontrol agent [146, 147].

19.8 Policy and plans in the import and export of EPF

Though there are almost 170 strains of mycopesticides formulated and available for commercial use, some exotic pests that are not indigenous to that country will be effectively controlled by such a specific biocontrol agent present in that exotic area itself. In such cases where the existing biocontrol agents fail to act effectively, considering the pest status and its impacts on yield, importing/exporting of specific biocontrol agents can be encouraged. Certain rules and guidelines for risk management related to the export, shipment, import, and release of biocontrol agents and other beneficial organisms have been laid out. These rules and guidelines are called International Standards for Phytosanitary Measures (ISPMs), which are available on the International Phytosanitary Portal (IPP). It lists the related responsibilities of contracting parties to the International Plant Protection Convention (IPPC), Convention on Biological Diversity (CBD), National Plant Protection Organization (NPPO), or other responsible authorities, importers, and exporters. The scope of these standards do not include living modified organisms, issues related to registration of biopesticides, or microbial agents intended for vertebrate pest control. These contents and data are taken from the ISPM 3 guidelines of the Food and Agricultural Organization of the United Nations for the export, shipment, import, and release of biological control agents and other beneficial organisms produced by the International Plant Protection Convention (IPPC) [141].

19.9 Background of IPPC

The setting up of IPPC was based on the need for a common and effective action to prevent the introduction and spread of pests and for promotion of control measures. In this context, the provisions of IPPC extend to any organism that is capable of harboring or spreading plant pests, mainly where international transportation is involved (Article 1). Section 4.1 of ISPM 20 (*Guidelines for a phytosanitary import regulatory system*) contains a reference to the regulation of biological control agents. It states: Imported commodities that may be regulated include articles that may be infested or contaminated with regulated pests. Examples of regulated articles are pests and biological control agents. Phytosanitary concerns may include the possibility that newly introduced biological control agents may primarily affect other non-target organisms. It may be a potential pest itself; it may act as a carrier/pathway for pests, hyperparasitoid, and hyperpathogens. In this sense, they may be regulated articles according to Article VII.1 of IPPC and ISPM20. In addition, the IPPC considers globally accepted environmental standards (Preamble), and contracting parties should also examine the potential for more significant environmental implications from releasing biological control agents and other beneficial creatures (for example, impacts on non-target invertebrates).

19.10 Purpose of the standard and some important standards

The objective of the standard is

- To ease the safe shipment, import, export, and release of biocontrol agents by providing guidelines, mainly through the development of national legislation where it does not exist.
- To describe the need for cooperation between importing and exporting countries so that benefits can be obtained with minimal adverse effects.
- To promote practices that will ensure safe and efficient use while minimizing environmental risks due to improper handling.

Some important standards are:

ISPM 2 – Framework for pest risk analysis.
ISPM 3 – Code of conduct for import and release of exotic biocontrol agents.
ISPM 11 – Pest risk analysis for quarantine pests (this includes pest risk assessment in relation to environmental risks, and this aspect covers environmental concerns related to the use of biological control agents).
ISPM 19 – Guidelines on lists of regulated pests.
ISPM 20 – Guidelines for a phytosanitary import regulatory system.

19.11 Requirements

The basic requirements for import and export Entomopathogenic fungi were already framed and proposed by the International Plant Protection Convention in 2005 and the document was published in 2017. The basic requirements involved in importing and exporting entomopathogenic fungi are mentioned in Figure 19.9.

19.12 Designation of responsible authority and description of general responsibilities

19.12.1 Contracting parties

Contracting parties should designate an authority with appropriate competencies (usually their NPPO) to be responsible for export certification and regulate the import or release of biological control agents and other beneficial organisms, subject to relevant phytosanitary measures and procedures.

Figure 19.9: Basic requirements involved in importing and exporting of entomopathogenic fungi.

19.12.2 General responsibilities

NPPO or other responsible authorities should access and audit the import/export documentation; should carry out a pest risk analysis before import or release of biological control agents, labeling; should ensure that biocontrol agents are taken directly to either quarantine station or mass rearing unit; and should also consider possible impacts on the environment.

19.12.3 Pest risk analysis

This analysis is required to estimate the likelihood or successful invasion of a plant pest, hyperparasitoid, and specificity toward a target pest that may occur due to the import/export of biocontrol agents. In addition, the analysis is required to assess the potential impact and also the options to mitigate. This step helps the decision on the control of import/export.

19.12.4 Responsibility of contracting parties

19.12.4.1 Importing country's responsibility

If the biological control agent or another beneficial organism already exists in the country, regulation may be required solely to guarantee that the organism is not contaminated or infested; and to ensure that interbreeding with indigenous genotypes of the same species does not introduce new phytosanitary hazards. For these reasons, inundative release may be limited. Documentaries about the organism's biology, origin, distribution, ecology, economic value, environmental impacts, and other enemies should all be kept on hand.

19.12.4.2 Exporting country's responsibility

If the biological control agent or other beneficial organism is already present in the country, regulation may only be needed to ensure there is no contamination or infestation of this organism or that interbreeding with local genotypes of the same species does not result in new phytosanitary risks. Inundative release may be restricted for these reasons.

19.12.4.3 Documents related to the potential hazard and contingency plans

Before the first importation, the importer should provide the documents related to the potential health hazard and risks posed to staff operatives while handling. NPPO or other responsible authority develops or adopts contingency plans or procedures. When the problem is identified, they should consider the possible emergency actions and implement them in an appropriate situation.

19.12.4.4 Release

NPPO/responsible authority should analyze the release requirement only in a specific area. In addition, they should have sufficient documents to allow traceback of the released biocontrol agents.

19.12.4.5 Monitoring and evaluation

The authorities should evaluate and respond to the impact on the target and nontarget organisms.

19.12.4.6 Communication

It is recommended that the NPPO or other responsible authority ensure that local users and suppliers of biological control agents or other beneficial organisms, farmers, farmer organizations, and other stakeholders are kept sufficiently informed and educated on the appropriate measures for their use.

19.12.4.7 Reporting

The contracting party should abide by any reporting obligations under the IPPC; for example, where an organism used as a biological control agent or beneficial organism has shown pest characteristics.

19.13 Authorities, acts, and conventions in India

9.1. National Biodiversity Authority (NBA): It is a statutory autonomous body under the Ministry of Environment, Forests and Climate Change, Government of India, established in 2003 to implement the provisions under the Biological Diversity Act, 2002, after India signed the Convention on Biological Diversity in 1992.

9.2. Salient provisions related to biocontrol agents in Biological Diversity Act, 2002.

Section 3: All foreign nationals require NBA approval to obtain Biological Resources.

Section 4: Indian individuals/entities to seek approval before transferring knowledge/research and material to foreigners.

Section 6: Prior approval of NBA before applying for any IPR based on research conducted on biological material and/or associated knowledge obtained from India.

9.3. The Cartagena protocol: The Biosafety Protocol under CBD (Convention on Biological Diversity) requires parties to make decisions on the import of LMOs for intentional introduction into the environment for risk assessments (Article 15). Risk assessment in Annexure III. Parties are also required to take measures to prevent unintentional transboundary movements.

9.4. The Nagoya–Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety: The Supplementary Protocol also includes provisions about civil liability.

19.14 Future prospects

In general, our knowledge of fungal entomopathogens has moved from simple observations of fungus killing insects to a determined attempt to learn how to employ these organisms as biological control agents. Most previous studies on endophytic fungi and other associated investigations have concentrated on co-culturing methods in an in vitro dual plate experiment to assess endophytic fungi's antagonistic effects against key targets. Anti-pest proteins produced by recombinant endophytic microbes can successfully invade the host employed in insect pest management [142]. However, it is to be noted that recombinant endophytic fungi with increased virulence can be a risk to pollinators and other beneficial insects. Therefore, the integrated use of EPF, such as *B. bassiana*, in combination with other chemical pesticides, has been investigated. According to certain scientists, the integration of various entomopathogens might aid in improving resistance management tactics and reducing ecosystem damage caused by the overuse of inorganic pesticides [143]. According to a study, one of the significant areas to be concentrated on is the ecology of fungal entomopathogens. Though there have been some significant developments in this part [123], in-depth knowledge of the most common genera of fungal entomopathogens should reveal the significant insights that make our understanding better so that it can be employed in an ecological approach for biological control. Some scientists advocated that several research areas should be used to understand fungal entomopathogens better [123]. Still, there are many other areas in need of research; for example, the impact of climate change on fungal entomopathogens and spore surface properties of fungi. All studies should include most entomopathogenic species and not only about *M. anisopliae* and *B. bassiana*. These investigations should give us new knowledge about manufacturing, storage, maintainance and utilization of fungal entomopathogens in the field. Further studies can also focus on the following:

i. Plant-microbe associations for stress tolerance and adaptation
ii. Symbiosis effect on plant's secondary metabolism
iii. Symbiosis impact on secondary metabolism of microbes
iv. The use of metagenomics and bioinformatics tools to determine entomopathogens diversity and phylogeny [144].

Important information on pathogenicity, genes, proteins, metabolites, and genetic linkages will be revealed by this endeavour. Furthermore, including EPF into IPM systems entails a thorough understanding of the abiotic and biotic factors that determine EPF's insecticidal effect and endophytic activity. Apart from that, to establish effective management techniques, it is critical to evaluate inoculation methods for extended colonization [145]. Even though biopesticides account for just 3% of the crop protection market worldwide currently, they are consistently expanding at

the rate of 10% each year. Mycoinsecticide comes in second (27 percent) in the worldwide biopesticide market, next to *Bacillus thuringiensis* products [10].

19.15 Conclusion

Biopesticides, based on entomopathogenic fungi, are safe and effective in managing insect pests. However, environmental and food safety issues drive the use of entomopathogenic fungus in the myco-biocontrol of insects. Also, contamination with mycotoxins (aflatoxins, trichothecenes, zearalenone, fumonisins, citrinin, etc.) that are generated by saprophytic fungi cannot be ruled out. Currently, much research is being done on entomopathogenic fungus identification and characterization. However, it is critical to find new EPF strains and commercialize those that have previously shown efficiency against certain insects. The current pathogenesis mechanism of entomopathogens is sluggish and has to be improved. Modern molecular biology approaches may alter the beneficial features of this fungus to increase field activity. The ecology of entomopathogens must also be better understood to create sensible techniques for enhancing their efficacy in field applications. It is also exciting to see biotechnology being used, but it should be more rigorous. A multi-faceted approach using all available integrated pest management (IPM) techniques offers a higher chance of controlling pests affordably. Hence, entomopathogenic fungi will likely become a major IPM component. The future of EPF-based biopesticides in IPM relies on scientists and other stakeholders working together.

Work contribution

G. Venkatesh, Writing – first draft; **P. Sakthi Priya**, Writing – first draft; **V. Anithaa**, Writing – first draft; **G. K. Dinesh**, Data Visualization, Software, Writing – first draft, review and editing; **S. Vel Murugan**, Writing – first draft; **Abinaya S.**, Writing – review and editing; **P. Karthika**, Writing – first draft, review, and editing; **P. Sivasakthivelan**, Writing – review and editing; **R. Soni**, Writing – review and editing; **A. Thennarasi,** Writing – review and editing

References

[1] Lokeshwari RK, Shantibala T. A review on the fascinating world of insect resources: Reason for thoughts. Psyche (Stuttg) 2010, 2010, 207570.

[2] Mwamburi LA. Beauvaria. In: Amaresan N, Senthil Kumar M, Annapurna K, Kumar K, Sankaranarayanan A-BT-BM in A.-E. eds. Beneficial microbes in agro-ecology. Amsterdam, Academic Press, Elsevier, 2020, 727–748.

[3] Bamisile BS, Akutse KS, Siddiqui JA, Xu Y. Model application of entomopathogenic fungi as alternatives to chemical pesticides: Prospects, challenges, and insights for next-generation sustainable agriculture. Frontiers of Plant Science 2021, 12. doi:10.3389/fpls.2021.741804.
[4] Bahadur A. Entomopathogens: Role of insect pest management in crops. Trends Hortic 2018, 1. doi:10.24294/th.v1i4.833.
[5] Vega FE, Meyling NV, Luangsa-ard JJ, Blackwell M. Insect pathology. In: Vega FE, Kaya HKBT-IP. 2nd edn, San Diego, Academic Press, 2012, 171–220.
[6] Hibbett DS, Binder M, Bischoff JF et al. A higher-level phylogenetic classification of the Fungi. Mycological Research 2007, 111, 509–547.
[7] St. Leger RJ, Wang C. Genetic engineering of fungal biocontrol agents to achieve greater efficacy against insect pests. Applied Microbiology and Biotechnology 2010, 85, 901–907.
[8] Batta YA. Control of main stored grain insects with new formulations of entamopathogenic fungi in diatomaceous earth dusts. International Journal of Food Engineering 2008, 4, 1–16.
[9] Ramanujam B, Poornesha B, Yatish KR, Renuka S. Evaluation of pathogenicity of different isolates of *Metarhizium anisopliae* sorokin against maize stem borer, *Chilo partellus* using laboratory bioassays. Biopesticides International 2015, 11, 89–95.
[10] Um M, Zakaria D, Galadima IB, Gambo FM, Maina UM, Zakaria D. A review on the use of entomopathogenic fungi in the management of insect pests of field crops. Journal of Entomology and Zoology Studies 2018, 6, 27–32.
[11] Abdelgany TM. Entomopathogenic fungi and their role in biological control. Omi. Gr. eBooks 2015, 46.
[12] Alexopoulos CJ, Mims CW, Blackwell M. Introductory mycology. New York, John Wiley & Sons, 1996.
[13] Mora MAE, Castilho AMC, Fraga ME. Classification and infection mechanism of entomopathogenic fungi. Arquivos Do Instituto Biologico (Sao. Paulo) 2018, 84. doi:10.1590/1808-1657000552015.
[14] Araújo JPM, Hughes DP. Diversity of entomopathogenic fungi. Which groups conquered the insect body? Advances in Genetics 2016, 94, 1–39.
[15] Ramanujam B, Rangeshwaran R, Sivakumar G, Mohan M, Yandigeri MS. Management of insect pests by microorganisms. Proc. Indian Natl. Sci. Acad. 2014, 80, 455–471.
[16] Glare T. Entamopathogenic fungi and their role in regulation of insect populations. Insect Control Biological Synthetic Agent 2010, 6, 387–419.
[17] Shah PA, Pell JK. Entomopathogenic fungi as biological control agents. Applied Microbiology and Biotechnology 2003, 61, 413–423.
[18] Hatai K, Roza D, Nakayama T. Identification of lower fungi isolated from larvae of mangrove crab *Scylla Serrate* in Indonesia. Mycoscience 2000, 41(6), 565–572.
[19] Tanada Y, Kaya HK. Insect pathology. Amsterdam, Acadenic Press, 2012, 665.
[20] Abdelaziz O, Senoussi MM, Oufroukh A et al. Pathogenicity of three entomopathogenic fungi to the aphid species, *Metopolophium dirhodum* Walker (Hemiptera: Aphididae) and their alkaline protease activities. Egyptian Journal of Biological Pest Control 2018, 28, 1–5.
[21] Torres Acosta RI, Humber RA, Sánchez-Peña SR. *Zoophthora radicans* (Entomophthorales), a fungal pathogen of *Bagrada hilaris* and *Bactericera cockerelli* (Hemiptera: Pentatomidae and Triozidae): Prevalence, pathogenicity, and interplay of environmental influence, morphology, and sequence data on fungal identification. Journal of Invertebrate Pathology 2016, 139, 82–91.
[22] Aung MO, Kang JC, Liang ZQ, Soytong K, Hyde KD. A new entamopathogenic species *Hymenostilbe furcata* parasitic on a hemipteran nymph in Northern Thailand. Mycotaxon 2006, 97, 241–245.

[23] Bensch K, Groenewald JZ, Dijksterhuis M et al. Species and ecological diversity within the *Cladosporium cladosporoides* (Davidiellaceae, Capnodiales). Studies in Mycology 2010, 67, 1–94.
[24] Ben FI, Boukhris-Bouhachem S, Eilenberg J, Allagui MB, Jensen AB. The occurrence of two species of entomophthorales (entomophthoromycota), pathogens of *Sitobion avenae* and *Myzus persicae* (Hemiptera: Aphididae), in Tunisia. BioMed Research International 2013, 2013. doi:10.1155/2013/838145.
[25] Kepler RM, Luangsa-Ard JJ, Hywel-Jones NL et al. A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). IMA Fungus 2017, 8, 335–353.
[26] Lacey LA, Frutos R, Kaya HK, Vail P. Insect pathogens as biological control agents: Do they have a future? Biological Control 2001, 21, 230–248.
[27] Lacey LA. Manual of techniques in insect pathology. Amsterdam, Academic press, 1997.
[28] Luangsa-ard JJ, Mongkolsamrit S, Thanakitpipattana D, Khonsanit A, Tasanathai K, Noisripoom W, Humber RA. Clavicipitaceous entamopathogens: New species in Metarhizium and a new genus Nigelia. Mycological Progress 2017, 16, 369–391.
[29] Luangsa-Ard J, Houbraken J, van Doorn T, Hong SB, Borman AM, Hywel-Jones NL, Samson RA. Purpureocillium, a new genus for the medically important *Paecilomyces lilacinus*. FEMS Microbiology Letters 2011, 321, 141–149.
[30] Mains EB. The genus Gibellula on spiders in North America. Mycologia 1950, 42, 306–321.
[31] Sanchez-Pena SR, Peña S. In vitro production of hyphae of the grasshopper pathogen Entomophaga grylli (Zygomycota: Entomophthorales): Potential for production of conidia. Florida Entomol 2005, 88, 332.
[32] Rumbos CI, Mendoza A, Kiewnick S. Effect of *Paecilomyces lilacinus* strain 251 on the survival and virulence of entamopathogenic nematodes under laboratory conditions. Nematologia Mediterranea 2007, 35, 103–107.
[33] Soper RS, Shimazu M, Humber RA, Ramos ME, Hajek AE. Isolation and characterization of entomophaga *Maimaiga* Sp. Nov., a fungal pathogen of gypsy moth *Lymantria Dispar* from Japan. Journal of Invertebrate Pathology 1988, 51(3), 229–241.
[34] Taheri-Talesh N, Horio T, Araujo-Bazán L et al. The tip growth apparatus of *Aspergillus nidulans*. Molecular Biology of the Cell 2008, 19, 1439–1449.
[35] Wynns AA, Jensen AB, Eilenberg J, James R. *Ascosphaera subglobosa*, a new spore cyst fungus from North America associated with the solitary bee *Megachile rotundata*. Mycologia 2012, 104, 108–114.
[36] Zekeya N, Mtambo M, Ramasamy S, Chacha M, Ndakidemi PA, Mbega ER. First record of an entomopathogenic fungus of tomato leafminer, *Tuta absoluta* (Meyrick) in Tanzania. Biocontrol Science and Technology 2019, 29, 626–637.
[37] Zimmermann G. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*): Biology, ecology and use in biological control. Biocontrol Science and Technology 2008, 18, 865–901.
[38] Tanabe Y, O'Donnell K, Saikawa M, Sugiyama J. Molecular phylogeny of parasitic Zygomycota (Dimargaritales, Zoopagales) based on nuclear small subunit ribosomal DNA sequences. Molecular Phylogenetics and Evolution 2000, 16, 253–262.
[39] Glare TR, Milner RJ. Ecology of entomopathogenic fungi. In: Arora DK, Ajello L, Mukerji KG. 2nd edn, New York, Marcel Dekker, 1991, 547–612.
[40] Samson RA, Evans HC, Latge JP. Atlas of entamopathogenic fungi. Heidelberg, Springer Berlin, 1998.
[41] Deacon JW. Modern mycology. Cambridge, Blackwell Science ltd, 1997.
[42] Bischoff JF, Rehner SA, Humber RA. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia 2009, 101, 512–530.

[43] Lefeuvre P, Calmes C, Reynaud B, Nibouche S, Costet L. Description and phylogenetic placement of *Beauveria hoplocheli* sp. nov., used in the biological control of the sugarcane white grub, *Hoplochelus marginalis* in Reunion Island. Mycologia 2015, 107, 1221–1232.
[44] Barr DJS. Chytridiomycota. In: Systematics and evolution. Berlin, Heidelberg, Springer, 2001, 93–112.
[45] De Faria MR, Wraight SP. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Contro 2007, 43, 237–256.
[46] Rehner SA, Buckley EP. A Beauveria phylogeny inferred from nuclear ITS and EF1-alpha sequences evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia 2005, 97, 84–98.
[47] Rehner SA, Posada E, Buckley P, Infante F, Castillo A, Vega E. Phylogenetic origins of African and neotropical *Beauveria bassiana* pathogens of the coffee berry borer, *Hypothenemus hampei*. Journal of Invertebrate Pathology 2006, 93, 11–21.
[48] Wraight S, Jackson M, De Kock S. Fungi as biocontrol agents: progress, problems and potential. Central Agricultural Biosecurity International 2001, 253–287.
[49] Shah PA, Pell JK. Entomopathogenic fungi as biological control agents. Applied Microbiology and Biotechnology 2003, 61, 413–423.
[50] Zhang SL, He LM, Chen X, Hueng B. B*eauveria lii* sp. nov., isolated from *Henosepilachna vigintiopunctata*. Mycotaxon 2012, 121, 199–206.
[51] Chen MJ, Huang B, Li ZZ, Spatafora JW. Morphological and genetic characterisation of *Beauveria sinensis* sp. nov, from China. Mycotaxon 2013, 124, 301–308.
[52] Agrawal Y, Mual P, Shenoy BD. Multi gene genealogies reveal cryptic species *Beauveria rudraprayagi* sp. nov. from India. Mycosphere 2014, 5, 719–736.
[53] Zimmermann G. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Science and Technology 2007, 17, 879–920.
[54] Milner JR. Current status of Metarhizium as a mycoinsecticide in Australia. Biocontrol News Information 2000, 21, 47–50.
[55] Mishra J, Tewari S, Singh S, Arora NK. Biopesticides: Where We Stand? In: Arora N, eds. Plant Microbes Symbiosis: Applied Facets. New Delhi, Springer. 2015. https://doi.org/10.1007/978-81-322-2068-8_2.
[56] Kachhawa D. Microorganism as a biopesticides. Journal of Entomology and Zoology Studies 2017, 5, 468–473.
[57] Rajula J, Rahman A, Krutmuang P. Entomopahogenic fungi in southeast Asia and Africa and their possible adoption in biological control. Biological Contro 2020, 151, 104–399.
[58] Ujjan AA, Shahzad S. Use of entomopathogenic fungi for the control of mustard aphids (*Lipaphis erysimi*) on canola(*Brassica napus*). Pakistan Journal of Botany 2012, 44, 2081–2086.
[59] Weng Q, Zhang X, Chen W, Hu Q. Secondary metabolites and the risks of *Isaria fumosorosea* and *Isaria farinose*. Molecules 2019, 24, 664.
[60] Clarkson JM, Charnley AK. New insights into the mechanisms of fungal pathogenesis in insects. Trends in Microbiology 1996, 4, 197–203.
[61] Hajek AE, St. Leger RJ. Interactions between fungal pathogens and insect hosts. Annual Review of Entomology 1994, 39, 293–322.
[62] Sinha KK, Choudhary AK, Kumari P. Entomopathogenic fungi. 2016.
[63] Ortiz-Urquiza A, Keyhani NO. Action on the surface: Entomopathogenic fungi versus the insect cuticle. Insects 2013, 4, 357–374.

[64] Gabarty A, Salem HM, Fouda MA, Abas AA, Ibrahim AA. Pathogencity induced by the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in *Agrotis ipsilon* (Hufn.). Journal of Radiation Research and Applied Sciences 2014, 7, 95–100.
[65] Cho EM, Kirkland BH, Holder DJ, Keyhani NO. Phage display cDNA cloning and expression analysis of hydrophobins from the entomopathogenic fungus *Beauveria (Cordyceps) bassiana*. Microbiology 2007, 153, 3438–3447.
[66] Boucias DG, Pendland JC, Latge JP. Nonspecific factors involved in attachment of entomopathogenic Deuteromycetes to host insect cuticle. Applied and Environmental Microbiology 1988, 54, 1795–1805.
[67] Gao Q, Jin K, Ying H et al. Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. PLoS Genetics 2011, 7. doi:10.1371/journal.pgen.1001264.
[68] Wang C, St. Leger RJ. The MAD1 adhesion of Metarhizium anisopliae links adhesion with blastospore production and virulence to insects, and the MAD2 adhesin enables attachment to plants. Eukaryotic Cells 2007, 6, 808–816.
[69] St.Leger RJ, Butt TM, Goettel MS, Staples RC, Roberts DW. Production in vitro of appressoria by the entomopathogenic fungus *Metarhizium anisopliae*. Experimental Mycology 1989, 13, 274–288.
[70] Napolitano R, Juárez MP. Entomopathogenous fungi degrade epicuticular hydrocarbons of *Triatoma infestans*. Archives of Biochemistry and Biophysics 1997, 344, 208–214.
[71] Hackman RH. Biology of the Integument. Berlin, Heidelberg, Springer, 1984, 583–610.
[72] Zacharuk RY. Penetration of the cuticular layers of elaterid larvae (Coleoptera) by the fungus *Metarrhizium anisopliae*, and notes on a bacterial invasion. Journal of Invertebrate Pathology 1973, 21, 101–106.
[73] Smith RJ, Grula EA. Toxic components on the larval surface of the corn earworm *(Heliothis zea*) and their effects on germination and growth of *Beauveria bassiana*. Journal of Invertebrate Pathology 1982, 39, 15–22.
[74] Sánchez-Pérez LDC, Barranco-Florido JE, Rodríguez-Navarro S, Cervantes-Mayagoitia JF, Ramos-López MÁ. Enzymes of entomopathogenic fungi, advances and insights. Advanced Enzyme Research 2014, 02, 65–76.
[75] Beli WJ. Comprehensive insect physiology, biochemistry and pharmacology. The International Journal of Biochemistry 1985, 17, 1281–1282.
[76] Pedrini N, Zhang S, Juárez MP, Keyhani NO. Molecular characterization and expression analysis of a suite of cytochrome P450 enzymes implicated in insect hydrocarbon degradation in the entomopathogenic fungus *Beauveria bassiana*. Microbiology 2010, 156, 2549–2557.
[77] Pedrini N, Ortiz-Urquiza A, Huarte-Bonnet C, Zhang S, Keyhani NO. Targeting of insect epicuticular lipids by the entomopathogenic fungus *Beauveria bassiana*: Hydrocarbon oxidation within the context of a host-pathogen interaction. Frontiers in Microbiology 2013, 4, 1–18.
[78] Dillon RJ, Charnley AK. Inhibition of *Metarhizium anisopliae* by the gut bacterial flora of the desert locust, *Schistocerca gregaria*: Evidence for an antifungal toxin. Journal of Invertebrate Pathology 1986, 47, 350–360.
[79] Eyal J, Fischbein AMKL, Grace R. Assessment of *Beauveria bassiana* which produces a red pigment for microbial control. World Journal of Microbiology and Biotechnology 1994, 44, 2263–2268.
[80] Bradfisch GA, Harmer SL. ω-Conotoxin GVIA and nifedipine inhibit the depolarizing action of the fungal metabolite, destruxin B on muscle from the tobacco budworm (*Heliothis virescens*). Toxicon 1990, 28, 1249–1254.

[81] Suzuki A, Taguchi H, Tamura S. Isolation and structure elucidation of three new insecticidal cyclodepsipeptides, destruxins C and D and desmethyldestruxin B, produced by *metarrhizium anisopliae*. Agricultural and Biological Chemistry 1970, 34, 813–816.
[82] Kodaira Y. Toxic substances to insects, produced by *aspergillus ochraceus* and *oopsra destructor*. Agricultural and Biological Chemistry 1961, 25, 261–262.
[83] Kodaira Y. Studies on the new toxic substances to insects, destruxin a and b, produced by *oospora destructor*. Agricultural and Biological Chemistry 1962, 26, 36–42.
[84] Samuels RI, Reynolds SE, Charnley AK. Calcium channel activation of insect muscle by destruxins, insecticidal compounds produced by the entomopathogenic fungus *Metarhizium anisopliae*. Comparative Biochemistry and Physiology Part C: Comparative 1988, 90, 403–412.
[85] Ebel RE, Lardy HA. Stimulation of rat liver mitochondrial adenosine triphosphatase by anions. The Journal of Biological Chemistry 1975, 250, 191–196.
[86] Gelardi M, Angelini C, Costanzo F, Dovana F, Ortiz-Santana B, Vizzini A. *Neoboletus antillanus* sp. nov. (Boletaceae), first report of a red-pored bolete from the dominican republic and insights on the genus neoboletus. MycoKeys 2019, 49, 73–97.
[87] Mannino MC, Huarte-Bonnet C, Davyt-Colo B, Pedrini N. Is the insect cuticle the only entry gate for fungal infection? Insights into alternative modes of action of entomopathogenic fungi. Journal of Fungi 2019, 5, 33.
[88] Schabel HG. Oral infection of Hylobius pales by *Metarrhizium anisopliae*. Journal of Invertebrate Pathology 1976, 27, 377–383.
[89] Rafaluk-Mohr C, Wagner S, Joop G. Cryptic changes in immune response and fitness in *Tribolium castaneum* as a consequence of coevolution with *Beauveria bassiana*. Journal of Invertebrate Pathology 2018, 152, 1–7.
[90] Biswas T, Joop G, Rafaluk-Mohr C. Cross-resistance: A consequence of bi-partite host-parasite coevolution. Insects 2018, 9. doi:10.3390/insects9010028.
[91] Batta YA. Efficacy of two species of entomopathogenic fungi against the stored-grain pest, sitophilus granarius l. (curculionidae: Coleoptera), via oral ingestion. Egyptian Journal of Biological Pest Control 2018, 28, 1–8.
[92] Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J et al. The faces of fungi database: Fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 2015, 74, 3–18.
[92] Lord JC, Hartzer K, Toutges M, Oppert B. Evaluation of quantitative PCR reference genes for gene expression studies in *Tribolium castaneum* after fungal challenge. Journal of Microbiological Methods 2010, 80, 219–221.
[94] Keilin D. On a new *Saccharomycete monosporella unicuspidata* gen. n. nom., n.sp., parasitic in the body cavity of a dipterous larva (d*asyhelea obscura winnertz*). Parasitology 1920, 12, 83–91.
[95] Van Der Veen KJ, Willebrands AF. Isoenzymes of creatine phosphokinase in tissue extracts and in normal and pathological sera. Clinica Chimica Acta 1966, 13, 312–316.
[96] Peirson HB. The life history and control of the pales weevil (*Hylobius Pales*). Petersham, Harvard Forest, 1921.
[97] Pedrini N. Molecular interactions between entomopathogenic fungi (Hypocreales) and their insect host: Perspectives from stressful cuticle and hemolymph battlefields and the potential of dual RNA sequencing for future studies. Fungal Biology 2018, 122, 538–545.
[98] Lacey CM, Lacey LA, Roberts DR. Route of invasion and histopathology of *Metarhizium anisopliae* in *Culex quinquefasciatus*. Journal of Invertebrate Pathology 1988, 52, 108–118.
[99] Goettel MS. Viability of *Tolypocladium cylindrosporum* (Hyphomycetes) conidia following ingestion and excretion by larval *Aedes aegypti*. Journal of Invertebrate Pathology 1988, 51, 275–277.

[100] Miranpuri GS, Khachatourians GG. Infection sites of the entomopathogenic fungus *Beauveria bassiana* in the larvae of the mosquito *Aedes aegypti*. Entomologia Experimentalis Et Applicata 1991, 59, 19–27.
[101 Butt TM, Coates CJ, Dubovskiy IM, Ratcliffe NA. Entomopathogenic Fungi: New insights into host-pathogen interactions. Advances in Genetics 2016, 94, 307–364.
[102] Sweeney AW. Infection of mosquito larvae by *Culicinomyces* sp. through anal papillae. Journal of Invertebrate Pathology 1979, 33, 249–251.
[103] Rodrigues J, Campos VC, Humber RA, Luz C. Efficacy of Culicinomyces spp. against *Aedes aegypti* eggs, larvae and adults. Journal of Invertebrate Pathology 2018, 157, 104–111.
[104] Rodrigues ML, Casadevall A. A two-way road: Novel roles for fungal extracellular vesicles. Molecular Microbiology 2018, 110, 11–15.
[105] Rodrigues J, Luz C, Humber RA. New insights into the in vitro development and virulence of *Culicinomyces sp*p. as fungal pathogens of *Aedes aegypti*. Journal of Invertebrate Pathology 2017, 146, 7–13.
[106] Xiao G, Ying SH, Zheng P et al. Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. Scientific Reports 2012, 2. doi:10.1038/srep00483.
[107] Hu X, Xiao G, Zheng P. Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. Proceedings of the National Academy of Sciences of the United States of America 2014, 111, 16796–16801.
[108] Bartlett MC, Jaronski ST. Mass Production of Entomogenous Fungi for Biological Control of insects. In: Burge MN, ed. Fungi in Biological Control Systems. Manchester UK, Manchester University Press, 1988, 61–85.
[109] Jaronski ST. Mass production of entomopathogenic fungi: State of the art. Sydney, USA. 2013.
[110] Lopes RB, Faria M, Glare TR. A nonconventional two-stage fermentation system for the production of aerial conidia of entomopathogenic fungi utilizing surface tension. Journal of Applied Microbiology 2019, 126, 155–164.
[111] Santos P, Abati K, Mendoza NVR, Mascarin GM, Delalibera Júnior I. Nutritional impact of low-cost substrates on biphasic fermentation for conidia production of the fungal biopesticide *Metarhizium anisopliae*. Bioresource Technology Reports 2021, 13, 100619.
[112] Pham TA, Kim JJ, Kim K. Optimization of solid-state fermentation for improved conidia production of B*eauveria bassiana* as a Mycoinsecticide. Mycobiology 2010, 38, 137.
[113] McWhorter CG. Pesticide Formulations. Journal of Environmental Quality 1974, 3, 94–95.
[114] Gasic S, Tanovic B. Biopesticide formulations, possibility of application and future trends. Pesticidi I Fitomedicina 2013, 28, 97–102.
[115] IRAC. Technical monograph no 2. 6th ed, Catalogue of pesticide formulation types and international coding system 2008.
[116] McWhorter CG. Pesticide Formulations. Journal of Environmental Quality 1974, 3, 94–95.
[117] Eilenberg J, Hajek A, Lomer C. Suggestions for unifying the terminology in biological control. Bio Control 2001, 46, 387–400.
[118] Dara SK, Montalva C, Barta M. Microbial control of invasive forest pests with entomopathogenic fungi: A review of the current situation. Insects 2019, 10. doi:10.3390/insects10100341.
[119] Samways MJ. Classical biological control and insect conservation: Arethey compatible? Environmental Conservation 1988, 15, 349–354.
[120] Hajek AE, Elkinton JS, Witcosky JJ. Introduction and spread of the fungal pathogen Entomophaga maimaiga (Zygomycetes: Entomophthorales) along the leading edge of gypsy moth (Lepidoptera: Lymantriidae) spread. Environmental Entomology 1996, 25, 1235–1247.

[121] Gautam RD. Biological pest suppression. New Delhi, Westvile publication house, 1994.
[122] Khan S, Guo L, Maimaiti Y, Mijit M, Qiu D. Entomopathogenic fungi as microbial biocontrol agent. Molecular Plant Breeding 2012. doi:10.5376/mpb.2012.03.0007.
[123 Vega FE, Goettel MS, Blackwell M et al. Fungal entomopathogens: New insights on their ecology. Fungal Ecology 2009, 2, 149–159.
[124] Glare TR, Milner RJ. Handbooks of applied mycology. Volume 2: Humans, animals and insects. In: Arora DK, Ajello L, Mukerj KG, ed. New York, Marcel Dekker, 1991, 547–612.
[125] Vestergaard S, Cherry A, Keller S, Goettel M. Environmental impacts of microbial insecticides. Dordrecht, Springer Netherlands, 2003, 35–62.
[126] Otieno WA. Microbial control of pest and plant diseases 1970–1980. In: Burges, ed. London, Academic Press, 1981.
[127] Zimmermann G. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology 2007, 17, 553–596.
[128] Steinhaus EA. Microbial diseases of insects. Annual Review of Microbiology 1957, 11, 165–182.
[129] Ignoffo CM, Hostetter DL, Sikorowski PP, Sutter G, Brooks WM. Inactivation of representative species of entomopathogenic viruses, a bacterium, fungus, and protozoan by an ultraviolet light source. Environmental Entomology 1977, 6, 411–415.
[130] Goettel MS, Douglas Inglis G. Manual of techniques in insect pathology. New York, Elsevier, 1997, 213–249.
[131] Goettel MS, Hajek AE, Siegel JP, Evans HC. In: Butt TM, Jackson C, Magan N, ed. Fungi as biocontrol agents: Progress, problems and potential. Wallingford, CAB International, 2001, 347–376.
[132] Henke MO, de Hoog GS, Gross U, Zimmermann G, Kraemer D, Weig M. Human deep tissue infection with an entomopathogenic *Beauveria* species. Journal of Clinical Microbiology 2002, 40, 2698–2702.
[133] Semalulu SS, MacPherson JM, Schiefer HB, Khachatourians GG. Pathogenicity of *Beauveria bassiana* in Mice. Journal of Veterinary Medicine Series B 1992, 39, 81–90.
[134] Strasser H, Vey A, Butt TM. Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of metarhizium, tolypocladium and beauveria species? Biocontrol Science and Technology 2000, 10, 717–735.
[135] Hu G, St. Leger RJ. Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) Reveal that it is rhizosphere competent. Applied and Environmental Microbiology 2002, 68, 6383–6387.
[136] Schaerffenberg B. Untersuchungen über die Wirkung der Insektentötenden PilzeBeauveria bassiana (Bals. Vuill.) undMetarrhizium anisopliae (Metsch.) Sorok. auf Warmblütler. Entomophaga 1968, 13, 175–182.
[137] El-Kadi MK, Xará LS, De Matos PF, Da Rocha JVN, De Oliveira DP. Effects of the Entomopathogen *Metarhizium anisopliae* on Guinea Pigs and Mice 1. Environmental Entomology 1983, 12, 37–42.
[138] Little LM, Shadduck JA. Pathogenesis of rotavirus infection in mice. Infection and Immunity 1982, 38, 755–763.
[139] Ferron P. Biological control of insect pests by entomogenous fungi. 1978.
[140] Copping LG. The manual of biocontrol agents. Alton, U.K, British crop protection council, 2004.
[141] International Plant Protection Convention. Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms. Rome, Italy, 2017.
[142] Fadiji AE, Babalola OO. Exploring the potentialities of beneficial endophytes for improved plant growth. Saudi Journal of Biological Sciences 2020, 27, 3622–3633.

[143] Aguilar-Marcelino L et al. Formation, resistance, and pathogenicity of fungal biofilms: Current trends and future challenges. In: Liliana Aguilar-Marcelino, Laith Khalil Tawfeeq Al-Ani, Filippe Elias de Freitas Soares, André Luís Elias Moreira, Maura Téllez-Téllez, Gloria Sarahi Castañeda-Ramírez, Ma. de Lourdes Acosta-Urdapilleta, Gerardo Díaz-Godínez, Jesús Antonio Pineda-Alegria, eds. Recent trends in mycological research. Cham, Springer, 2021, 411–438.

[144] Fadiji AE, Ayangbenro AS, Babalola OO. Unveiling the putative functional genes present in root-associated endophytic microbiome from maize plant using the shotgun approach. Journal of Applied Genetics 2021, 62, 339–351.

[145] Mantzoukas S, Eliopoulos PA. Endophytic entomopathogenic fungi: A valuable biological control tool against plant pests. Applied Science 2020, 10. doi:10.3390/app10010360.

[146] Singh D, Kour Raina T, Singh J. Entomopathogenic fungi: An effective biocontrol agent for management of insect populations naturally. Journal of Pharmaceutical Sciences Research 2017, 9(6), 883.

[147] Bamisile BS, Siddiqui JA, Akutse KS, Aguila LCR, Xu Y. General limitations to endophytic entomopathogenic fungi use as plant growth promoters, pests and pathogens biocontrol agents. Plants 2021, 10. doi:10.3390/plants10102119.